The University of Chicago

STUDIES OF THE ADRENAL GLANDS OF THE DOG IN VITAMIN B_1 DEFICIENCY

A PART OF A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY
1940

By
JULIA E. GOODSELL

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WEIGHT CHANGES IN THE CORTEX AND THE MEDULLA OF THE ADRENAL GLAND OF THE DOG IN ACUTE VITAMIN-B_1 DEFICIENCY

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Numerous investigators have shown that there are many pathological conditions in which the adrenals hypertrophy while other endocrine glands atrophy. Outstanding among the conditions in which this enlargement is reported to occur are injections of hormones, avitaminoses, inanition and infection (1–6). Histological changes in both the cortex and medulla have been described, but there is no clear cut evidence indicating whether these changes consistently involve the cortex, the medulla or both. From the variety of changes which have been reported it seems probable that the part of the gland affected may depend upon either the nature or the severity of the pathological condition. Evidence for this is given by Deansley (6) who made a study, not only of the histological picture, but also of the volume changes in the cortex and medulla of the adrenals in mice when given injections of thyroxine, morphine or killed B. gaertner.

Although many workers have reported enlargement of the adrenals in vitamin-B_1 deficiency, data are lacking in regard to the extent of the involvement of each part of the gland. Sure (4) has reported a total enlargement of the adrenals in rats in vitamin-B_1 deficiency which was 50 per cent greater than that of the controls kept at the same plane of nutrition.

In this study an attempt has been made to determine weight changes in both parts of the gland in acute vitamin-B_1 deficiency in the dog so that the portion responsible for the increased weight may be known.

METHODS. Seven young mature dogs weighing from 5 to 14 kgm. were used. Each dog was wormed, immunized against distemper, and maintained on a normal diet for at least one week before the deficient ration was started. This ration, which was fed until acute symptoms of vitamin-B_1 deficiency appeared, consisted of:

	Per cent
Casein	19
Sucrose	60

	Per cent
Cottonseed oil	8
Salt mixture (186)*	4
Autoclaved yeast	8
Agar	1
Cod liver oil concentrate†	

* Manganese and copper added.

† Squibbs Adex Tablets—1 tablet per day.

The ration was given ad libitum, the average daily consumption during the first week on the diet being 150 grams. After this time the food consumption became more variable. Five to six days before the appearance of acute symptoms the food intake averaged approximately 30 grams. Table 1 shows the initial and final body weights of each dog and the time required to produce severe symptoms of polyneuritis in each case (36–59 days). Prior to the onset of acute symptoms frequent attacks of vomiting occurred. Three to four days later the dogs became extremely spastic and presented the characteristic symptoms of acute B_1 deficiency. All dogs were allowed to live 24 hours after the appearance of these symptoms, then killed, and the adrenals removed immediately.

Determination of weights of the cortex and medulla. In order to save the cortex of the glands for physiological tests the following procedure was devised for determining the weights of the cortex and the medulla. Each pair of glands was dissected free of connective tissue and fat in a moist chamber and weighed in a weighing bottle. Each gland was cut in half longitudinally and the medulla, which stood out clearly from the cortex, was carefully teased out by blunt dissection. All of the adrenal glands removed from the vitamin-B_1 deficient dogs as well as from the controls were weighed in this manner (tables 1 and 2).

To establish the range of variation of these ratios in normal dogs, the adrenals from 96 dogs obtained from the pound were dissected and weighed. Each dog was classified as to approximate age (immature, mature, or old), sex, general health, and, in the case of females, its stage in the estrual cycle. All dogs were weighed immediately after being killed.

Baker (7) has shown that the weight of the adrenal glands varies with the age and the weight of the animal and that the ratio of medulla weight to cortex weight is approximately the same in mature males as in females in dioestrus. Hence, as a basis for comparison, only those dogs were chosen that were healthy, mature, in dioestrus, in the case of females, and within the same weight range as those in the series of B_1 deficient animals (table 2). All of the data were analyzed statistically for significance of difference, using a formula for small samples given by Tippett (8).

Results and discussion. A common method used to indicate the size of an organ is to express it as a per cent of the body weight. Com-

parison of the percentage organ weights for the vitamin-B_1 deficient animals with those for normal animals will indicate whether any change in size has occurred. As a loss of body weight usually accompanies vitamin-B_1 deficiency, the question arises whether the initial or the final body weight should be used in making these computations. Even though there is no actual change in size of an organ, if a marked loss of body weight occurred during the experimental period, the values obtained by expressing the organ weight as a per cent of the *final* body weight will be larger than those for normal animals.

As McCarrison (1) and Jackson (5) have shown that the loss of body weight in the various avitaminoses is due not only to a loss of body fat but also to atrophic changes in the organs and tissues, it should be expected that a loss of weight of the endocrine glands would also occur. Hence, the

TABLE 1

Showing weights of adrenal glands in relation to body weight in vitamin-B_1 deficient dogs

DOG NUMBER	SEX	DEPLETION TIME	INITIAL BODY WT.	FINAL BODY WT.	GLAND WT.	CORTEX WT.	MED. WT.	GLAND WT. PER CENT INITIAL BODY WT.	GLAND WT. PER CENT FINAL BODY WT.	CORTEX WT. PER CENT INITIAL BODY WT.	CORTEX WT. PER CENT FINAL BODY WT.	MED. WT. PER CENT INITIAL BODY WT.	$\frac{\text{CORTEX WT.}}{\text{MED. WT.}}$
		days	*kgm.*	*kgm.*	*gm.*	*gm.*	*gm.*						
1	F	36	7.26	8.35	0.922	0.856	0.066	0.013	0.011	0.012	0.010	0.0010	13.0
2	M	37	7.09	6.40	0.901	0.826	0.081	0.013	0.014	0.012	0.013	0.0011	10.2
3	M	39	5.38	5.87	0.941	0.895	0.046	0.017	0.016	0.017	0.015	0.0010	10.8
4	M	48	7.79	6.46	1.367	1.256	0.111	0.017	0.021	0.016	0.019	0.0014	11.3
5	M	49	8.87	6.89	0.991	0.902	0.089	0.012	0.014	0.010	0.013	0.0010	10.1
6	M	59	10.4	7.83	1.207	1.082	0.125	0.012	0.015	0.011	0.014	0.0012	8.6
7	M	55	13.9	8.49	1.649	1.521	0.128	0.012	0.019	0.011	0.018	0.0010	11.8

maintenance of the weight of a gland under conditions in which other organs are losing weight can be considered a *relative* hypertrophy. On the other hand, the hypertrophy can be considered as *absolute* if the weight of the gland, when expressed as a per cent of the *initial* body weight, is greater than that for normal animals.

In order to take both of these factors into consideration computations were made both on the basis of the initial and of the final body weights (table 3). It can be seen that the increase in size of the gland is due to the cortex, which undergoes an absolute (3A) as well as a relative (3B) hypertrophy.

On the other hand, the medulla weights of the adrenals of the B_1 deficient dogs, when expressed as a per cent of the initial body weight are lower than those for the normal animals, showing a tendency for this part of the gland

to follow the same trend as most of the organs and tissues (table 3A). For this reason the atrophy would not be apparent if the medulla weight

TABLE 2

Showing variation in weight of adrenal glands in relation to body weight in normal mature dogs

DOG NUMBER	SEX	BODY WT.	GLAND WT.	CORTEX WT.	MED. WT.	GLAND WT. PER CENT BODY WT.	CORTEX WT. PER CENT BODY WT.	MED. WT. PER CENT BODY WT.	CORTEX WT. / MED. WT.
		kgm.	*gm.*	*gm.*	*gm.*				
1	M	7.27	0.962	0.815	0.147	0.013	0.011	0.0021	5.5
2	M	8.60	1.060	0.929	0.131	0.011	0.011	0.0013	7.1
3	M	9.30	1.053	0.878	0.175	0.011	0.009	0.0019	5.0
4	M	12.70	1.190	0.988	0.202	0.009	0.008	0.0016	4.0
5	M	7.97	1.70	1.320	0.387	0.021	0.016	0.0040	3.4
6	M	12.20	1.510	1.340	0.176	0.012	0.015	0.0014	7.6
7	M	12.0	1.320	1.116	0.204	0.011	0.009	0.0017	5.5
8	M	8.80	0.903	0.689	0.214	0.010	0.008	0.0023	3.4
9	M	8.30	0.993	0.838	0.055	0.012	0.010	0.0066	15.1
10	M	7.27	1.146	0.991	0.155	0.016	0.014	0.0021	6.4
11	M	11.13	0.909	0.767	0.142	0.008	0.006	0.0014	5.4
12	M	7.38	0.789	0.722	0.067	0.010	0.010	0.0009	10.7
13	M	7.72	1.045	0.941	0.104	0.013	0.012	0.0013	9.3
14	M	11.37	0.954	0.817	0.137	0.008	0.007	0.0012	5.9
15	M	11.30	0.792	0.705	0.087	0.007	0.006	0.0008	8.1
16	M	9.10	0.715	0.635	0.080	0.008	0.009	0.0009	7.9
17	M	12.05	1.110	0.965	0.145	0.009	0.008	0.0012	6.6
18	M	12.50	1.220	1.030	0.190	0.010	0.008	0.0015	5.4
19	M	13.0	1.362	1.217	0.145	0.010	0.009	0.0011	8.4
20	M	12.50	1.169	0.968	0.201	0.009	0.008	0.0016	4.8
21	F	6.20	0.659	0.571	0.088	0.010	0.009	0.0014	6.5
22	F	9.70	1.219	1.076	0.143	0.013	0.011	0.0015	7.5
23	F	8.60	0.935	0.848	0.087	0.011	0.010	0.0010	9.8
24	F	8.80	1.30	1.113	0.187	0.015	0.013	0.0021	6.0
25	F	5.90	0.705	0.531	0.174	0.012	0.009	0.0030	3.3
26	F	7.72	0.950	0.786	0.164	0.012	0.010	0.0021	4.8
27	F	7.50	0.932	0.786	0.146	0.012	0.010	0.0020	5.4
28	F	6.58	1.044	0.879	0.165	0.016	0.013	0.0025	5.3
29	F	9.09	1.382	1.197	0.185	0.015	0.013	0.0020	6.5
30	F	6.80	1.180	0.982	0.199	0.017	0.014	0.0030	4.9
31	F	10.11	1.029	0.915	0.114	0.010	0.009	0.0011	8.0
32	F	8.40	1.115	0.930	0.184	0.013	0.011	0.0022	5.0
33	F	11.37	1.012	0.878	0.134	0.009	0.008	0.0012	6.6

were expressed as a per cent of the *final* body weight, as the loss in total body weight would mask the loss in weight of the medulla.

An increase is also noted in the ratio of the weights of cortex to medulla

(table 3B). Baker (7) from a comprehensive study of the weights of the adrenals of 1250 dogs reported that the weights of cortex to medulla were approximately 5 to 1 in both sexes. The results for normal dogs obtained in this study showed average ratios of 6 to 1. Considering the comparatively crude method employed, that the cortex might be saved for further work, this ratio corresponds well with Baker's. In the B_1 deficient dogs the average ratio for weight of cortex to medulla was 10.8 to 1 which is significantly higher than that obtained for the normal dogs. It can be seen that the increase in the ratio could be due either to an increase in cortex weight or to a decrease in medulla weight. From the results shown in table 3B it seems probable that both factors are involved.

TABLE 3

Comparison of adrenal glands of normal and of vitamin-B_1 deficient dogs

A. Expressed as per cent initial body weight

	NUMBER OF DOGS	GLAND WEIGHT PER CENT	STAN. DEV.	t	p	CORTEX WEIGHT PER CENT	STAN. DEV.	t	p	MED. WEIGHT PER CENT	STAN. DEV.	t	p
B_1 def.	7	0.014	0.0024			0.0125	0.0024			0.0011	0.0002		
				2.41	0.04			2.42	0.04			2.4	0.04
Normal	33	0.011	0.0025			0.0101	0.0023			0.0017	0.0006		

B. Expressed as per cent final body weight

	NUMBER OF DOGS	GLAND WEIGHT PER CENT	STAN. DEV.	t	p	CORTEX WEIGHT PER CENT	STAN. DEV.	t	p	CORTEX / MEDULLA	STAN. DEV.	t	p
B_1 def.	7	0.016	0.0030			0.0145	0.0028			10.8	1.29		
				4.0	0.01			4.2	0.01			5.1	0.01
Normal	33	0.011	0.0025			0.0101	0.0023			6.0	2.36		

As the results presented above indicate that the cortex is responsible for the enlargement of the adrenal glands in vitamin-B_1 deficiency in the dog, the question arises whether this necessarily means that there is an increased secretory activity of this part of the gland. Some workers have reported histological changes in the cortex under various pathological conditions which they have interpreted as an indication of increased activity. But as yet there is no direct evidence to justify this assumption. In a paper to follow, an attempt has been made to correlate weight changes of the cortex in vitamin-B_1 deficiency with changes in activity as indicated by a biological test.

SUMMARY

1. The adrenal cortex undergoes both a relative and an absolute hypertrophy in acute vitamin-B_1 deficiency in the dog.

2. The adrenal medulla shows a tendency to atrophy.

3. The ratio of the weight of cortex to medulla in normal dogs was found to be 6 to 1 whereas in the vitamin-B_1 deficient dogs it was 10.8 to 1.

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CHANGES IN CONCENTRATION OF STEROID COMPOUNDS IN THE ADRENAL CORTEX OF THE DOG IN VITAMIN-B_1 DEFICIENCY AS INDICATED BY THE BITTERLING TEST

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One of the outstanding changes described in the adrenal cortex in vitamin-B_1 deficiency is the change in the distribution of lipoids. McCarrison (1) and Kellaway (2), on the basis of histological studies, reported a wider distribution of the lipoids in the cortex in avian beri beri. Findlay (3) found a similar picture in the rat. That this change might conceivably be related to functional activity seems probable, as the various compounds which have been isolated from the cortex and shown to maintain life in an adrenalectomized animal are steroid compounds and would therefore be contained in the lipoid fraction of the gland. Zwemer (4), who made a study of adrenal morphology in various animals under both normal and pathological conditions, relates the wider distribution of lipoids to increased activity of the gland. Hoerr (5), on the other hand, does not think there is any good morphological evidence for indicating the function of the adrenal lipoids. Whitehead (6, 7) has shown that species variation must be considered in interpreting changes in cortical lipoids, and that the distribution changes with sex and age. As it is debatable whether changes in morphology of the gland should be used to gauge its functional state a physiological approach to the problem seemed desirable.

In 1936 Barnes, Kantner and Klawans (8) reported that aside from the gonads the adrenal glands were the only tissue which would yield an extract causing a lengthening of the ovipositor of the Japanese bitterling. Other investigators have shown that this reaction can be produced at will by various androgens and estrogens, and also by pure corticosterone (9 to 13). These results suggest that the bitterling test, though not specific for any one steroid compound, can be used to indicate the presence of this particular group of compounds. As this test can be made roughly quantitative it might serve as a useful criterion of the functional state of the adrenal glands until the identity of the true cortical hormone (or hormones) has been established.

Methods. A. *Measurement of ovipositor lengths.* Kleiner, Weisman

and Mishkind (14) and DeWit (11, 15) determined the extent of elongation of the ovipositor of the bitterling by visual comparison with the length of the rays of the anal fin. Since the ovipositor may elongate to a distance two or three times the length of these rays this method is only suitable for detecting gross changes. In order to make the more accurate measurements necessary for quantitative study, an objective record of ovipositor lengths was obtained by photographing the ovipositor against a millimeter ruler at constant position and magnification (2×). This was done by holding the fish in a glass compartment against the side of a square glass container. The negatives were projected onto a sheet of paper (enlargement 100×), and the outline of the ovipositor traced. This outline was then marked off with a marking wheel and the ovipositor length recorded in terms of the number of spacings made by the wheel. By photographing the ovipositor of one fish ten times and measuring each negative by this method the standard deviation was 4.3 (based on an average of 131 spacings).

To eliminate the element of variability in initial ovipositor lengths all data are expressed as per cent lengthenings over the initial lengths; in each case the initial length was determined prior to placing the fish in the solution. During the experimental period the length was determined at approximately 12-hour intervals. By using a running-water bath the temperature was maintained between 17° and 19°C. throughout the time these experiments were carried out (Jan. to May, 1940).

B. *Preparation of lipoids from the adrenal cortex.* The glands used to test the bitterling response were obtained from the dogs of the previous investigation, on which measurements of the weight changes in acute vitamin-B_1 deficiency had been made. The cortex of the gland, which had been dissected away from the medulla, was thoroughly ground with ether at room temperature. Ten cubic centimeters of ether per gram of tissue was used and the grinding continued for ten minutes. This procedure was carried out three times to insure complete extraction of the lipoids. Aliquots representing known amounts of cortex were pipetted into liter beakers. After evaporating to dryness the extract was suspended in 100 cc. of water at 55°C. and the solution made up to 500 cc. with water from the aquarium. Two fish were placed in each beaker after the solutions had reached the temperature of the water in the aquarium.

C. *Standardization of the bitterling response.* In order to establish differences in the concentration of the active compounds in different glands it was necessary to work below the concentration yielding a maximal lengthening. Barnes et al. (8) reported that the extract from 0.75 to 1.0 gram of adrenal cortex per liter of water was necessary to produce lengthening of the ovipositor. In some preliminary experiments, performed during the fall months to check their results, it was found that a concentration of

less than a gram of cortex per liter of water failed to produce consistent lengthening in all of the fish treated. Consequently this was the lowest concentration which could be used at this time. In order to determine whether concentrations greater than 1:1000 would produce proportionately greater lengthening, solutions were prepared with the aliquots representing one, two and three grams of cortex, each suspended in 1000 cc. of water. Four fish were treated with each concentration. It can be seen from figure 1 that there is less difference between the 3:1000 and the 2:1000 concentrations than between the 2:1000 and the 1:1000 concentrations. Thus the 3:1000 is approaching the concentration that will produce a maximal response. With the 1:1000 concentration not only is the total lengthening less but also a regression of the ovipositors begins at the end of 48 hours. No regression is noted in the ovipositors of the fish treated with the two higher concentrations even at the end of 66 hours. Treatment was stopped after this period of time because previous experiments had shown that if the fish were left longer in solutions of high concentrations, many of them died. On the basis of these observations the 1:1000 concentration was considered sufficiently below the maximal concentration and was used in the experiments performed during the month of February, 1940.

As Kleiner et al. (9) reported a change in sensitivity of the fish with the approach of the breeding season (March to August), a second standardization of the bitterling response was made in March, 1940, before beginning the assay of adrenal cortical steroids from the second group of dogs. Concentrations of 1:1000 and 1:2000 were used, six fish being treated with each concentration. Since these lower concentrations were not injurious to the fish, treatment was continued for 90 hours in order to obtain a more complete picture of the regression of the ovipositors. The results given in figure 2 show that the 1:2000 concentration produced a lengthening of the ovipositors of approximately the same magnitude as the 1:1000 concentration had produced in the previous standardization. And again there were differences both in the per cent lengthening of the ovipositors and in the time at which regression occurred. Since the 1:1000 concentration produced considerably greater elongation in this month than in the preceding month it was thought advisable to use the lower concentration in subsequent experiments.

In assaying the cortical extract from the normal and the vitamin-B_1 deficient dogs the per cent lengthenings of similar groups of fish treated during the same time interval were compared. This seemed preferable to comparing responses of the same group of fish treated first with extract from normal dogs and then a month later with that from B_1 deficient animals for the following reasons: 1, no fish could be treated oftener than once a month, as previous experiments had shown less consistent responses when tests were run at more frequent intervals; 2, the death of any of the

fish would alter the character of the groups, and 3, the change in age and condition of the fish would cause changes in sensitivity to the extract.

Results. *Group I.* In February, 1940, 11 fish were treated with the extracts of the adrenal cortex from three dogs in which acute symptoms of vitamin-B_1 deficiency had been produced (table 1). During the same month, ten fish were treated with extracts from the adrenals of normal dogs. In figure 3 the average per cent lengthenings of the groups of fish are plotted against time. Approximately a 43 per cent increase in ovipositor lengthening is noted in the fish treated with the extract from the adrenals of the B_1 deficient dogs over that obtained in the fish treated with the extract from the glands of normal dogs.

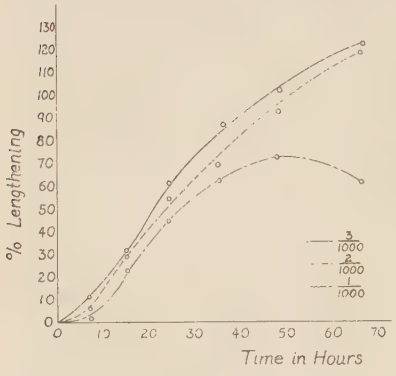

Fig. 1

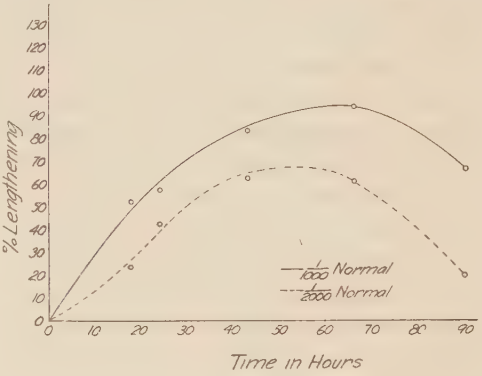

Fig. 2

Fig. 1. Bitterling response, February, 1940, to different concentrations of extract of adrenal cortex from normal dogs.

Fig. 2. Bitterling response, March, 1940, to different concentrations of extract of adrenal cortex from normal dogs.

Group II. In March, 1940, the responses of 15 fish treated with extracts from the adrenals of two dogs suffering from acute vitamin-B_1 deficiency were compared with those of 20 fish treated with extracts from the glands of normal dogs (fig. 4). Again, there is a much greater lengthening of the ovipositors of the fish treated with the extract from the adrenals of the B_1 deficient animals (about 50 per cent greater lengthening than in the controls).

Group III. Eighteen fish were treated with the extracts from the adrenals of two B_1 deficient dogs at the end of April, 1940. During the same time interval 17 fish were treated with the extracts from the glands of normal dogs. Results are shown in figure 5. The difference between the maximal per cent lengthening is not as great as in the two former groups (10 per cent). The smaller difference may have resulted from a further change in sensitivity of the fish due to approach of the breeding

season. This theory is borne out by the progressive increase in per cent lengthening of the ovipositors of the fish treated with the extract from the glands of normal dogs in successive months (table 1). If the sensitivity had increased, the concentration of 1:2000 of the extract from the adrenals of normal dogs was not sufficiently below that concentration necessary to evoke a maximal response and may even have been sufficient to produce maximal responses in some of the fish. However, the onset of regression of the ovipositors was later than in the controls, a factor which was shown by the standardization to vary with the concentration of extract used, and one which can be used to make reliable comparisons.

Comment. In analyzing the data statistically two factors have been considered: 1, the maximum per cent lengthening, and 2, the per cent

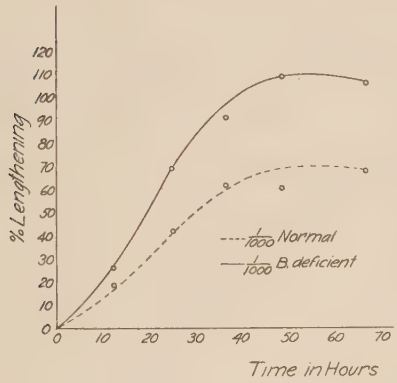

Fig. 3

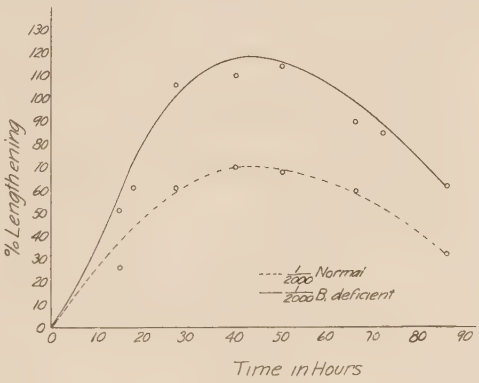

Fig. 4

Fig. 3. Bitterling response, group I, February, 1940, to 1:1000 concentration of adrenal cortical extracts.

Fig. 4. Bitterling response, group II, March, 1940, to 1:2000 concentration of adrenal cortical extracts.

lengthening at the end of 60 hours. Analysis of the results (Tippett, 16) based on the maximum per cent lengthening of the ovipositors shows that in groups I and II there are significant differences between the responses of the fish treated with the extract from the adrenals of the B_1 deficient dogs and those of the fish treated with the extract from the adrenals of normal dogs (table 1). In group III, which was run during April, the ten per cent difference over the normal in the maximum per cent lengthening of the ovipositors is not great enough to be statistically valid.

The second comparison, of the per cent lengthening of the ovipositors at the end of 60 hours, was chosen because regression of the ovipositors had begun in all of the fish by this time. In groups I and II this analysis gives almost identical results with those of the preceding method. By employing this method of analysis it is possible to validate the differences in the third group statistically (table 1). Thus a significant change in the

steroid compounds in the third group has been demonstrated in spite of the increased sensitivity of the fish. Due to a scarcity of fish it was impossible to carry out a third standardization at this time.

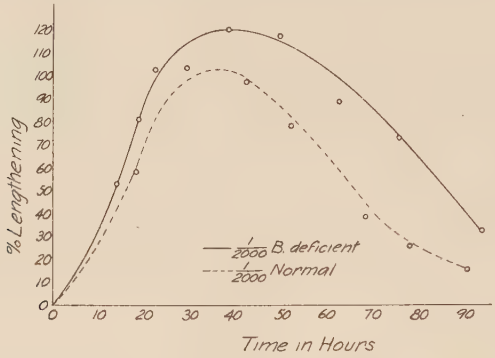

Fig. 5. Bitterling response, group III, April, 1940, to 1:2000 concentration of adrenal cortical extracts.

TABLE 1

Comparison of effects on bitterling response of extract of adrenals from normal and from vitamin-B_1 deficient dogs

GROUP	NUMBER OF DOGS	DEPLETION PERIOD	MONTH TREATED	CONCENTRATION OF SOLUTION	NUMBER OF FISH	MAXIMUM PER CENT LENGTH	STAN. DEV.	t	p	PER CENT LENGTH AT 60 HOURS	STAN. DEV.	t	p
		days											
I													
B_1 def.	3	37											
		38	Feb.	1:1000	11	115.0	19			105.0	26		
		40						4.0	0.01			3.6	0.01
Normal	2	0	Feb.	1:1000	10	79.0	19			69.0	15		
II													
B_1 def.	2	48											
		49	Mar.	1:2000	15	121.0	38			96.0	41		
								3.5	0.01			3.3	0.01
Normal	4	0	Mar.	1:2000	20	81.3	25			58.0	29		
III													
B_1 def.	2	55											
		59	Apr.	1:2000	18	126.0	27			89.0	38		
								1.0	0.3			2.68	0.02
Normal	3	0	Apr.	1:2000	17	116.0	34			57.0	29		

Discussion. As it has been demonstrated that the various steroid compounds isolated from the adrenal cortex vary considerably in respect to their ability to maintain the life of adrenalectomized animals, there is

the possibility that the greater potency of the extract from the adrenals of the B_1 deficient dogs may be due merely to a shift from a less active to a more active compound. However, from the results obtained in this investigation it seems most probable that the greater effect is due to an increase in concentration of these compounds in the adrenal cortex. The results from the standardization of the bitterlings showed that increased amounts of an identical extract produced greater elongation of the ovipositors. This would seem to be a concentration effect. That the effect is due to an increased concentration of the steroid compounds is also supported by histological evidence in which an increased lipoid content and a wider distribution of the lipoids throughout the cortex have been described. Finally, it has been shown (Goodsell, 1941) that the hypertrophy of the adrenal gland which occurs in acute vitamin-B_1 deficiency is due to an increase in size of the cortex. The results from this study demonstrate an accompanying increased potency of the steroid fraction. The simplest explanation of these related phenomena seems to be that an increased amount of the active substance has been produced.

SUMMARY

1. The use of the bitterling test as a sensitive biological method for a quantitative determination of the changes in the concentration of the steroid compounds in the adrenal cortex has been described.

2. By the use of this test it has been shown that the concentration of steroid compounds in the adrenal cortex was increased in dogs suffering from acute vitamin-B_1 deficiency.

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